

CLAIMS

What is claimed is:

1. An isolated nucleic acid molecule encoding a isoprenoid biosynthetic enzyme, selected from the group consisting of:
 - 5 (a) an isolated nucleic acid molecule encoding the amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18 and 24;
 - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 10 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS; and
 - (c) an isolated nucleic acid molecule that is complementary to (a) or (b).
2. The isolated nucleic acid molecule of Claim 1 selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23.
- 15 3. A polypeptide encoded by the isolated nucleic acid molecule of Claim 1.
4. The polypeptide of Claim 3 selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, and 18.
- 20 5. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 620 amino acids that has at least 60% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:2 or a second nucleotide sequence comprising the complement of 25 the first nucleotide sequence.
6. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 394 amino acids that has at least 55% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ 30 ID NO:4 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
7. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 231 amino acids that has at least 52% identity based on the Smith-Waterman method of alignment 35 when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

8. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 285 amino acids that has at least 50% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:8 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
9. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 157 amino acids that has at least 69% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:10 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
10. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 544 amino acids that has at least 67% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:12 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
11. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 297 amino acids that has at least 57% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:14 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
12. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 511 amino acids that has at least 34% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:16 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
13. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 497 amino acids that has at least 49% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:18 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

14. An isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 318 amino acids that has at least 65% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:24 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
- 5 15. A chimeric gene comprising the isolated nucleic acid molecule of any one of Claims 1 or 5-14 operably linked to suitable regulatory sequences.
- 10 16. A transformed host cell comprising the chimeric gene of Claim 15.
- 15 17. The transformed host cell of Claim 16 wherein the host cell is selected from the group consisting of bacteria, yeast, filamentous fungi, and green plants.
- 20 18. The transformed host cell of Claim 17 wherein the host cell is selected from the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*.
- 25 19. The transformed host cell of Claim 17 wherein the host cell is selected from the group consisting of soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops, sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.
- 30 20. A method of obtaining a nucleic acid molecule encoding an isoprenoid compound biosynthetic enzyme comprising:
- 35 (a) probing a genomic library with the nucleic acid molecule of any one of Claims 1 or 5-14;
- (b) identifying a DNA clone that hybridizes with the nucleic acid molecule of any one of Claims 1 or 5-14; and
- (c) sequencing the genomic fragment that comprises the clone identified in step (b),
- wherein the sequenced genomic fragment encodes an isoprenoid biosynthetic enzyme.

21. A method of obtaining a nucleic acid molecule encoding an isoprenoid biosynthetic enzyme comprising:

(a) synthesizing an at least one oligonucleotide primer corresponding to a portion of the sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23; and

(b) amplifying an insert present in a cloning vector using the oligonucleotide primer of step (a); wherein the amplified insert encodes a portion of an amino acid sequence encoding an isoprenoid biosynthetic enzyme.

10 22. The product of the method of Claims 20 or 21.

23. A method for the production of isoprenoid compounds comprising: contacting a transformed host cell under suitable growth conditions with an effective amount of a carbon source whereby an isoprenoid compound is produced, said transformed host cell comprising a set of nucleic acid molecules encoding SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 15 16, 18 and 24 under the control of suitable regulatory sequences.

24. A method according to Claim 23 wherein the transformed host cell is selected form the group consisting of *Aspergillus*, *Trichoderma*, *Saccharomyces*, *Pichia*, *Candida*, *Hansenula*, *Salmonella*, *Bacillus*, *Acinetobacter*, *Rhodococcus*, *Streptomyces*, *Escherichia*, *Pseudomonas*, *Methylomonas*, *Methylobacter*, *Alcaligenes*, *Synechocystis*, *Anabaena*, *Thiobacillus*, *Methanobacterium* and *Klebsiella*.

25 25. A method according to Claim 23 wherein said methanotrophic bacteria:

- (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme.

30 26. A method according to Claim 25 wherein said methanotrophic bacteria is *methylomonas* 16a ATCC PTA 2402.

27. A method according to Claim 23 wherein the transformed host cell is selected form the group consisting of soybean, rapeseed, sunflower, cotton, corn, tobacco, alfalfa, wheat, barley, oats, sorghum, rice, *Arabidopsis*, cruciferous vegetables, melons, carrots, celery, parsley, tomatoes, potatoes, strawberries, peanuts, grapes, grass seed crops,

sugar beets, sugar cane, beans, peas, rye, flax, hardwood trees, softwood trees, and forage grasses.

28. A method according to Claim 23 wherein the carbon source is selected from the group consisting of monosaccharides, oligosaccharides, polysaccharides, carbon dioxide, methanol, methane, formaldehyde, formate, and carbon-containing amines.

29. A method according to Claim 23 wherein the transformed host is selected from the group consisting of *Methylomonas*, *Methylobacter* and *Methanobacterium* and the carbon source is selected from the group consisting of methane and methanol.

30. A method of regulating isoprenoid biosynthesis in an organism comprising, over-expressing at least one isoprenoid gene selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17 and 23 in an organism such that the isoprenoid biosynthesis is altered in the organism.

31. A method according to Claim 30 wherein said isoprenoid gene is over-expressed on a multicopy plasmid.

32. A method according to Claim 30 wherein said isoprenoid gene is operably linked to an inducible or regulated promoter.

33. A method according to Claim 30 wherein said isoprenoid gene is expressed in antisense orientation.

34. A method according to Claim 30 wherein said isoprenoid gene is disrupted by insertion of foreign DNA into the coding region.

35. A mutated gene encoding a isoprenoid enzyme having an altered biological activity produced by a method comprising the steps of:

- (i) digesting a mixture of nucleotide sequences with restriction endonucleases wherein said mixture comprises:
 - a) a native isoprenoid gene;
 - b) a first population of nucleotide fragments which will hybridize to said native isoprenoid gene;
 - c) a second population of nucleotide fragments which will not hybridize to said native isoprenoid gene;

wherein a mixture of restriction fragments are produced;

- (ii) denaturing said mixture of restriction fragments;
- (iii) incubating the denatured said mixture of restriction fragments of step (ii) with a polymerase;

- (iv) repeating steps (ii) and (iii) wherein a mutated isoprenoid gene is produced encoding a protein having an altered biological activity.